Studies in the Experimental Analysis of Sex.

Part 8.—On the Effects of the Removal and Transplantation of the Gonad in the Frog (Rana fusca).

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With Plates 43–46.

THE experiments described in this paper were performed in the Department of Pathology, and we are indebted to Professor Dreyer for invaluable advice as to the best methods of carrying them out. The large material derived from the experiments and from normal animals was examined and worked up in the Department of Comparative Anatomy. Nussbaum, who made a series of parallel experiments some years ago, did not anæsthetise his animals, and recommends a special method for preventing the animal, when it is operated on, from blowing its lungs out into the abdominal incision. In all our experiments the frog was anæsthetised with ether, and it was then found unnecessary to take any further precautions. An opening was made into the abdominal cavity by a longitudinal incision on one side of the anterior abdominal vein, the testis or ovary of both sides was removed, and in certain cases a testis from the same animal, or from

[1] The experiments for this paper were performed by G. S. The chief part of the examination of the sections and all the drawings are the work of E. S.

another, was transplanted into the peritoneal cavity. The wound was then sutured together in two layers with cotton, and the animal was kept in a zinc box lined with damp moss, which was frequently renewed. A supply of small worms was given to the frogs, and these were eagerly devoured in the summer and early autumn, but during late autumn and winter the frogs refused to feed owing, no doubt, to the fact that normally during these months the frogs are hibernating and do not feed in a state of nature.

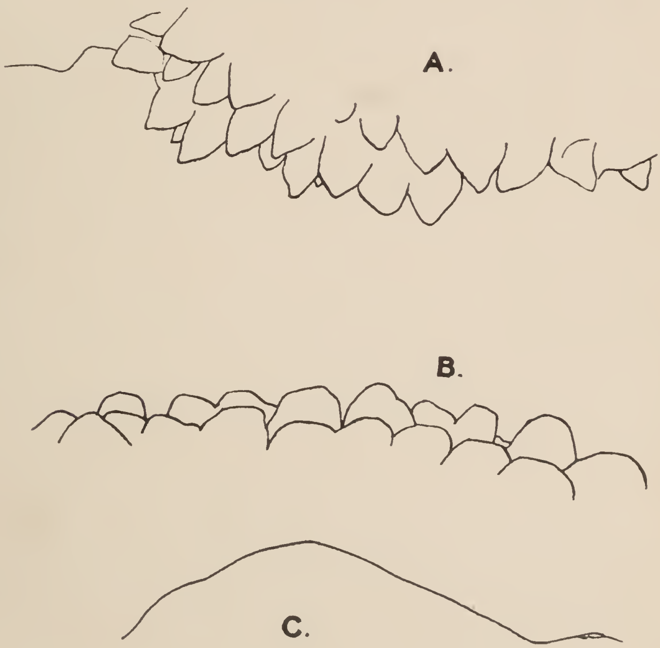
A great difference was observed in the mortality of the animals according as they were operated on in the summer or autumnal months, the mortality in spring and summer being very high, but in late autumn or winter very low. In one set of fifteen frogs operated upon in November, the mortality was 0 per cent.

The object of the experiments was to determine the effect of ovariotomy and castration upon the development of the thumb, which normally exhibits a characteristic pad in the male; to discover whether the effects of castration in the case of the male could be counterbalanced by the transplantation of testes from other frogs, or by the injection of testis-extract, and also to observe the fate of the transplanted organs according as they were left in the body of the animal to which they properly belonged (auto-transplantation), or transferred to the body of another individual, male or female (allo-transplantation).

Before describing the experiments it is desirable to give an account of the cyclical changes which take place in the testes and the thumb-pads of normal adult male frogs (R. fusca) at different times of year. Our own observations agree on the whole with those of Nussbaum (**1**) and Ploetz (**2**), but we must add certain facts with regard to the thumb which are of essential importance for the interpretation of experimental results. The testes exhibit their smallest size in March or April after the shedding of the spermatozoa at the breeding-season. From this period onwards until August they steadily increase in size, attaining their full dimensions by the beginning of September. From September until the breeding season in March no increase in size or alteration of the cellular structure occurs, the testes apparently remaining in a state of complete inactivity during this period. About March the shedding of the sperm normally occurs, and the cycle begins again. These alterations in size are accompanied by corresponding changes in the cellular activity of the organs. After the shedding of the spermatozoa at the breeding season the diminished testes consist of the germinal tubes lined with spermatogonia, and containing in their cavities remains of spermatozoa that have not been shed and the débris of the supporting cells to which the spermatozoa were attached in bundles. The spermatogonia soon begin to divide and proliferate, and to form groups of cells known as spermatocysts. The rapid increase of these spermatocysts, or nests of spermatogonia, continues through the summer months, and is responsible for the active growth of the testes. In June and July spermogenesis, or the formation of spermatocytes from the proliferating spermatogonia, is active, and from August to October the formation of ripe spermatozoa from spermatids is definitely finished. In November, December, January, and February the testes are crammed with ripe spermatozoa, and no apparent changes occur in the cellular structure or activities of the testes. We may, therefore, say that the activity of the testes coincides with the months of active feeding, namely, May to September, and that during the months when the frogs are hibernating at the bottom of a pond the testes remain in a fully mature and quite inactive state (see Table on p. 468).

The thumb-pads of the male frog also undergo a cyclical change, but their periods of growth and rest do not obviously correspond at all to those of the testes. Immediately after the breeding season the thumb-pads, with their deeply pigmented papillæ, are cast off, and the thumb remains comparatively smooth from late April or May until August or September. The comparative smallness of the pad at this period is due to the reduction of the glands and to the smooth, even surface from which the epidermal papillæ have almost entirely disappeared (fig. 32). Thus during the months when the most active growth of the testes is taking place the thumb-pads remain apparently inactive and smooth. In August and September the epidermal papillæ begin to be obvious, and from this time onwards until about February a continuous increase of the epidermal papillæ and pigmentation occurs. During the greater part of the time when the thumb-pads are attaining their characteristic rough and pigmented appearance the testes remain inactive and unchanged—a fact which has been too readily overlooked by writers on the correlation of the primary and secondary sexual characters. Although the above statement with regard to the thumb is roughly correct, a careful examination of the thumbs under a dissecting microscope of a series of male frogs throughout the year reveals much individual variability, and brings out one fact which is of prime importance in the interpretation of certain experimental results (see Table, p. 468). It will be noticed from the table, which refers to male frogs caught wild during the year December 5th, 1910–November 7th, 1911, that when the thumb-pad is thrown off after the breeding season in March or April, the thumb does not necessarily at once become perfectly smooth and unpapillated. Small, though quite marked, colourless papillæ may be retained on the pad, which denote the bases of the old prominent pigmented papillæ present in the breeding season (cf. Text-figs. A and B). It was not until June, July and August that the majority of male frogs had quite or nearly smooth pads (Text-fig. C), and even during these months small papillæ were observable in a certain number of specimens. During September all the frogs observed had begun to develop the papillæ again to a marked extent, and from this month onward the increase in size of the papillæ was continuous up to the breeding season. The disappearance of the papillæ after the breeding season is therefore not a sudden or complete disappearance, but takes place gradually and at different rates in different individuals during the summer months. It is perfectly true that after the breeding season there is a sudden casting of the very large black papillæ on the pad, but smaller papillæ remain below during the early summer months, and are only gradually obliterated. It appears, further, that this gradual obliteration of the small papillæ which takes place in the summer is not due to a reduction of the epidermal cells, but

TEXT-FIGS. A, B, AND C.



Camera drawings of outline of part of the thumb-pad of normal male frogs (side view). A. In breeding season. B. After shedding pigmented pad. C. Quite smooth, as in July and August. × 67.

to an increase of them over the whole pad, with the result that the spaces between the papillæ are filled up and a smooth surface is thereby formed. This interpretation—viz. that the obliteration of the remains of the papillæ during the summer is due, not to a reduction, but to an increase of the epidermal cells—is based on the fact that sections of the smooth thumbs in summer (July) show a thickened instead of a reduced epidermal covering (Pl. 46, fig. 32), the valleys between the papillæ being filled up with epidermal tissue. I is quite right to argue, therefore, that during the summer, when the thumbs are becoming smooth and the testes are actively growing, that the thumb tissue is not undergoing reduction, but that an actual proliferation of epidermal tissue is taking place. The fundamental importance of these facts will be apparent when we come to criticise the experimental results obtained by Nussbaum (see p. 459, et seq.).[1]

The above facts apply to the species R. fusca = temporaria, with which all the succeeding experiments have been performed. It appears that in R. esculenta the cyclical changes are not so regular, and it is said that it is impossible to induce this species to breed or to go through its normal cycle in captivity.

We will first of all give the results of our experiments in the transplantation of testes, and we will deal first with the cases of allo-transplantation, where the testis or testes of a male were transferred to the peritoneal cavity of a female frog.

Allo-transplantation of Testes into Female.

Seven successful experiments were performed, which may be considered in order according to the length of time the testes remained in the body of the female.

No. 1.—Female frog, with one testis from male frog B., implanted September 29th, 1910. Killed November 8th,

[1] Although it is customary to speak of the thumb-pad increasing in size during the winter months before the breeding system, there is really very great doubt whether the increase in the number and size of the papillæ which occurs then indicates any actual process of growth or increase in mass at all. There is an increase of the surface area of the pad owing to the folding involved in the formation of the papillæ, but this does not necessarily mean any actual growth or increase of mass. Although it is difficult to prove, it appears very probable that the whole of the actual increase in mass or growth of the thumb-pad really takes place in the summer months when the pad is smooth.

1910. Time in body of female, $1\frac{1}{4}$ months. Testis found attached in two places to bladder and ovary; a small vascular supply was present from capillaries of these organs. Sections show the presence of a considerable number of blood-vessels round the edge of the testis. The testis tubules show distinct signs of degeneration both in the ripe spermatozoa and in the spermatogonia lining the tubules, although they are still present. It has not undergone any marked reduction in size. There is no increase of fibrous tissue.

No. 2.—Female frog, with one testis from male frog A., implanted September 29th, 1910. Killed December 15th, 1910. Time in body of female, less than three months. Testis enclosed in a fold of peritoneum near the bladder, vascularised from superior abdominal vein and mesenteric blood-vessels. A band of fibrous tissue (Pl. 43, fig. 1, *fi.*) is conspicuous on the side of attachment of the testis to the host, which is probably invasive fibrous tissue derived from the host. There is marked degeneration of the spermatozoa in the tubules, many of them being broken up into irregular masses of chromatin, among which some phagocytes can be recognised which have engulfed the chromatin lumps derived from spermatozoa. The degeneration of the tubules has proceeded more rapidly towards the side of attachment than along the free edge. The majority of the spermatogonia have disappeared, but traces can still be seen chiefly round the periphery of the organ.

No. 3.—Female frog, ovaries removed, and two testes implanted September 30th, 1909. Died March 24th, 1910. Time in body, six months. Both testes found intact, attached to peritoneum, with blood supply from ovarian mesentery and anterior abdominal. In both testes (vide Pl. 43, fig. 2) the tubules are disintegrating, the spermatogonia have all disappeared, and the spermatozoa are broken down into masses and granules of chromatic material. Among the tubules are very numerous phagocytes (fig. 3), which appear to be devouring the chromatin clumps derived from the degenerate spermatozoa. In both testes there is a broad strip of fibrous tissue along the side by which attachment to the body of the host was effected. This fibrous tissue is continuous with strands of similar tissue ramifying between the testis tubules. It seems certain that the broad outer band of fibrous tissue is derived from the host, but the origin of the inner strands is doubtful, as they may be derived by a growth of the connective tissue which is normally present between the tubules.

The spermatogonia or germinal cells lining the tubules have completely disappeared.

No. 4.—Female frog, ovaries removed, and two testes from a male implanted October 8th, 1909. Killed April 7th, 1910. Time in body, six months. The two testes found attached to peritoneum with blood supply. The one testis is much larger (fig. 4) than the other (fig. 5). In both the clumps of spermatozoa are degenerate (fig. 6), and there is a great invasion of fibrous tissue, especially in the smaller testis. In the latter (fig. 7) the fibrous tissue has invaded the tubules, and granules of chromatin derived from broken-down spermatozoa are found lying in the fibrous network.

The spermatogonia have completely disappeared.

No. 5.—Female frog, ovaries removed, two testes from a male implanted October 10th, 1909. Died May 5th, 1910. Time in body, 7¾ months. Testes attached with vascularisation, one to peritoneum of body-wall, the other to the remains of the ovary. The microscopic appearance of these testes is very similar to those of No. 4. The invasion of fibrous tissue to replace the tubules and the degenerated spermatozoa has proceeded very far, and again is more advanced in one testis than in the other. Again, there are no traces of spermatogonia lining the tubules; they have entirely disappeared. The testes are much reduced in size.

No. 6.—Female frog, with one testis from male frog C, implanted September 30th, 1910, died August 11th, 1911. Time in body, 10½ months. Testis (fig. 8) has shrunk to a very small size, about one eighth of its original dimensions. It was attached to the body-wall, near the anterior abdominal vein. The microscopic structure shows that the greatly reduced testis now consists almost entirely of fibrous tissue, among which the scanty remains of the granules derived from the broken-up spermatozoa can be recognised in places (fig. 9). Beyond these scattered granules absolutely no remains of germinal tissue are left, the whole having been replaced by fibrous scar tissue.

Allo-Transplantation of Testes into Male.

Three successful experiments were performed of this kind, and the results agree completely with the foregoing, showing that the destruction of the adult spermatozoa and of the spermatogonia and their replacement by fibrous tissue, probably derived from the host, occurs equally when the testes are transferred to another male as when they are transferred to a female. Thus—

No. 7.—Male frog, with one testis from male B, implanted on September 29th, 1910. Killed on November 6th, 1910. Time in body $1\frac{1}{4}$ months. This experiment is exactly parallel to No. 1, in which the other testis from male B was placed into a female for the same period. The result in this case was quite similar. The testis was found reattached and vascularised (fig. 10). The spermatozoa, especially in the interior of the tubes, have undergone considerable degeneration, though some of the bundles are still intact (fig. 11). The spermatogonia have begun to disappear, leaving vacuoles round the edges of the tubules, but the germinal nuclei can still be recognised as present in small numbers.

No. 8.—Male frog, with one testis from male A, implanted on September 29th, 1910. Killed December 15th, 1910. Time in body $2\frac{1}{2}$ months. This is a parallel experiment to No. 2, and shows (fig. 12) about the same amount of degeneration and a considerable increase of fibrous tissue (cf. fig. 1). Phagocytosis of degenerate spermatozoa is proceeding. The spermatogonia have almost entirely disappeared, this degeneration having proceeded further than in No. 2.

No. 9.—Male frog, with one testis from male C, implanted on September 30th, 1910. Killed September 7th, 1911. Time in body, $11\frac{1}{4}$ months. This is a parallel experiment to No. 6, and again shows exactly the same result. The testis (fig. 13), which was attached to the bladder, consists, as in experiment No. 6, of a small mass of fibrous tissue enclosing a small amount of granular chromatic material, derived from broken-down spermatozoa that have not been completely absorbed (cf. fig. 8).

Auto-Transplantation of Testes in Male.

Two successful experiments were made, in which both testes were severed from their connections with the body and left loose in the peritoneal cavity of the same individual. Thus as regards vascular supply and connection with the body, the severed testes were in the same position as in the allo-transplantation experiments, the only difference being that they were implanted in the body to which they naturally belonged, instead of being transferred to another body. Nevertheless, as the experiments show, their fate is a very different one.

No. 10.—Male frog, in which both testes were severed from all connections and left in the peritoneal cavity October 10th, 1909. Killed March 25th, 1910. Time in body $5\frac{1}{2}$ months. Both testes were found reattached to peritoneum of body-wall, one highly vascularised (fig. 14), the other very slightly (fig. 15). The highly vascularised testis shows an enormous infiltration of blood, the blood sinuses (*bl. sp.*) surrounding the highly degenerate masses of spermatozoa (fig. 16). There is no phagocytosis to be observed. Round the edge of the testis are small tubules (figs. 16 and 17) lined with living spermatogonia of different sizes, which suggest a recent proliferation of the germinal cells. There is very little fibrous tissue. In the second testis the one side of the whole of the centre is composed of fibrous tissue (figs. 15 and 18), a part of which, at any rate, appears to be of a secondarily intrusive nature. At the tops, and round the edges in places, are tubules lined with living and actively proliferating spermatogonia (Pl. 45, fig. 19).

No. 11.—Male frog, in which both testes were severed from all connections and left in the peritoneal cavity October 4th, 1909. Killed March 30th, 1910. Time in body, 6 months. Both testes were found attached to peritoneum with very slight vascular supply. One testis (fig. 20) was much reduced in size, and surrounded by a thick fibrous layer containing a large amount of pigment. The inner part consists of fibrous tissue, while round the edge are tubules containing living spermatogonia (fig. 21). There are no traces of spermatozoa.

In the other testis (fig. 22), which is much larger, one end corresponds to the structure of the first testis, being composed of tubules with living spermatogonia and no spermatozoa, but with a considerable intrusion of fibrous tissue. At the other end are tubules with some spermatogonia and bunches of spermatozoa in the first stages of disintegration. In the centre of the testis are tubules with more completely degenerate spermatozoa and a certain amount of fibrous tissue. The vascularisation and connection of the testis with the body is at the upper end, where the spermatozoa have disappeared, and there is a large amount of fibrous tissue.

Results of Transplantation Experiments given above.

In all cases of allo-transplantation where the testes are transferred from the body of a male to the peritoneal cavity of another individual, whether male or female, the same processes of degeneration, absorption and replacement by fibrous tissue take place. In ten or eleven months the original testicular tissue has practically disappeared and nothing remains but a very small nodule of fibrous scar-tissue. In all cases the testis placed loose in the peritoneal cavity becomes reattached and vascularised. Within a month the ripe spermatozoa present in the tubules begin to break down into lumps and granules of chromatin which are attacked by phagocytes. The breaking down of the spermatozoa would appear to take place soonest in the region of greatest vascularisation. The germinal cells, or spermatogonia lining the tubules, begin to be absorbed and to disappear very rapidly; within six months there is no sign of them left. The gradual replacement of the degenerate tubules by fibrous tissue appears to take place chiefly by the ingrowth of invasive connecting tissue from the host at the region of attachment of the testis to the host's body. It is, however, impossible to be certain that some of the fibrous tissue in the centre of the testis is not derived from the connective tissue of the testis itself.

In the cases of auto-transplantation, where the testes were simply severed from all their connections and left in the peritoneal cavity of the animal to which they properly belonged, a marked difference was observed in that the spermatogonia, or young germinal cells lining the tubules, so far from degenerating, remained alive, and after about six months showed signs of active proliferation. The ripe spermatozoa, on the other hand, undergo degeneration and replacement by fibrous tissue. The origin of this fibrous tissue would appear to be less from the surrounding area of attachment outside the testis than from the fibrous connective-tissue of the testis itself.

We may compare these results with those obtained by other authors, especially by Foa (**5**) and Herlitzka (**6**), who both worked with Amphibia.

Foa found that in frogs, testes auto-transplanted below the skin in the lymph-sac do not adhere, but that when placed in the abdominal cavity they always reattached themselves, and became vascularised. All his experiments were apparently done as auto-transplantations, and did not last more than a month. He observed the partial substitution of fibrous for testicular tissue, but he makes no observations on the survival of the spermatogonia. His chief point is to prove the degeneration and disappearance of the ripe spermatozoa, in opposition to the earlier and very incomplete experiments of Mantegazza.

Herlitzka, using Tritons, transplanted the testes from one animal into the abdominal cavity of another, and his experiments lasted two months. At the end of this time he states that in both males and females the allo-transplanted testes were nearly completely replaced by fibrous tissue, which he believes to have been derived entirely from the host. He apparently did not make any auto-transplantations, so that though his results agree with our own, they do not throw any light upon the survival or destruction of the spermatogonia in the two cases.

C. R. Stockard (**11**) has recently made some experiments in which he transplanted the ovaries of guinea-pigs and salamanders onto various organs, e.g. the testis and liver of the male. He states that the ovaries when transplanted on to the testes "lived" better than on the liver. After forty-one days he finds no degeneration either of eggs or stroma or young follicles, but after seven months the ova cells can no longer be distinguished, the entire transplanted piece being now of the stroma type. From the figures given we can only conclude that there was a complete destruction of the germinal part of the allo-transplanted ovary and its replacement by connective-tissue stroma, a conclusion which Stockard himself does not seem to draw.

We may give a short summary of other work upon the transplantation of gonads in other branches of the Vertebrata.

Successful auto-transplantation of ovaries in the rabbit has been claimed by Knauer, Ribbert (**4**), Grigorieff, Rubinstein, and Halban, and all these authors claim that the presence of the auto-transplanted ovary stops the atrophy of the uterus.

Hunter and Berthold and Foges (**7**), for the cock, state that pieces of testis can be successfully auto-transplanted, and that the presence of such grafts in an abnormal situation may permit the development of the male secondary sexual characters, although the testes have been otherwise removed.

This has been fully confirmed by Shattock and Seligmann (**8**), who affirm the continued life of the germinal tissue.

According to Foges (**7**) the allo-transplantation of testes or pieces of testis from one animal into another invariably fails, the graft being absorbed.

Ribbert's observations led him to conclude that in the case of the thyroid auto-transplantation was successful, but allo-transplantation always ended in absorption. In his experiments with the auto-transplanted ovaries of guinea-pigs the ripe follicles discharged and atrophied, and after about fifty days new follicles grew and came to maturity with normal corpora lutea formation.

On the other hand, his auto-transplantations of guinea-pig testes were failures, complete degeneration setting in after twelve days, the epididymis alone remaining normal.

Marshall and Joly (**9**) carried out a series of auto- and allo-transplantations of the ovaries of rats, and their results show that the auto-transplanted grafts survived far better than the allo-transplantations; indeed, the disappearance of the germinal tissue in the latter cases seems to have been invariable. The allo-transplantations seem to have been more successful when performed upon closely related females.

On the whole, the above evidence strongly supports the fundamentally different results obtained in the case of auto- and allo-transplantation, in the former case the young germinal tissue surviving, in the latter being destroyed and absorbed.

We find it difficult to harmonise these and our own results with the experiments of Guthrie (**12**), in which he performed ovariotomy on hens, and grafted the ovaries of another breed into the operated hens. He claims that the allo-transplanted ovaries became functional and produced fertile eggs and chicks, which partook of the characters of the hen to which the ovary originally belonged. There are, however, two possibilities here. The complete removal of the ovary from a hen is a notoriously difficult operation, as the germinal tissue is closely adherent to the walls of the inferior vena cava. It is quite possible, therefore, that the fertile eggs produced by the operated hen were derived from the remains of the ovary, which had not been completely removed, and not from the ingrafted ovary. The fact that the chicks exhibited coloration characters similar to the bird from which the ingrafted ovary was obtained may possibly have been due to the male or female used in the experiment belonging to an impure strain of whites, which normally threw chicks with black coloration. Another possibility is that the ingrafted ovary contained some nearly ripe follicles which actually did come to maturity and gave rise to the fertilised eggs, but that the ovary subsequently degenerated.[1] Setting aside this experiment, the remarkable difference in the fate of transplanted gonads according as they are transferred to another animal or left in the body of the same animal may be considered further in its theoretical bearings. In both cases we have found that the mature, fully formed spermatozoa undergo destruction. The cause of this destruction seems to be connected with the abnormal vascularisation of the testis from a source other than the normal one. It would seem to indicate either that the ripe spermatozoa in the testis are not merely passively stored there, but that they require special substances in the nature of food-materials or oxygen, which they do not obtain from the abnormal vascular supply, or else that the abnormal vascularisation brings in blood with properties toxic to the spermatozoa. The latter explanation appears more reasonable from the following considerations. One of us has repeatedly observed in the case of the serum of a great number of animals (frogs, birds, and mammals) that the normal serum of an animal agglutinates and dissolves the spermatozoa derived from the same animal.

[1] Since the above was written, Davenport ('Journal of Morphology,' vol. xxii, No. 1, p. 111, 1911) has repeated Guthrie's experiments and finds that the engrafted ovary always degenerates, thus supporting the interpretation given above. We have not dealt here with the question of transplantation of gonads in Invertebrates, where the chance of success with allo-transplantations appears to be much greater (see the important papers of Meisenheimer, and more recently, Kopec [14]).

This process is exceedingly rapid in the case of the cock, less rapid in the rabbit, and comparatively slow in the frog. Now, if we suppose that the presence of the testis in an abnormal situation leads to inflammation and a rich vascularisation uncontrolled by the normal nervous supply, we can understand how the spermatozoa, even in an auto-transplanted testis, come to be damaged and destroyed, and the process, once begun, would continue with increasing magnitude as the presence of damaged spermatozoa with their débris led to further inflammation. The survival of the spermatogonia in the auto-transplanted testes and their destruction in the allo-transplantations indicate that the blood and body fluids of an individual are less toxic to its own embryonic cells than the blood of another individual, and also less toxic to its own spermatogonia than to its own fully formed spermatozoa.

The Effects of Ovariotomy, Castration, Transplantation, etc., on the Properties of the Thumb-pad.

We will, first of all, give the experiments on females which were designed to test whether the removal of the ovaries, with or without transplantation of testes or injection of testis extract, caused the thumb to swell or become rough and pigmented. Certain of the frogs used have already been referred to in preceding experiments, and they are referred to by the same numbers.

No. 3.—Female frog, ovaries removed and two testes implanted September 30th, 1909. February 10th, 1910. Testes pieces inserted in dorsal lymph-sac.

March 3rd, 1910.	Injected 1 c.c. testis extract.
March 6th, 1910.	Ditto.
March 10th, 1910.	Ditto.
March 14th, 1910.	Ditto.
March 18th, 1910.	Ditto.

The injections were made with sterile saline into the dorsal lymph sac; altogether the substance of twenty-three testes was injected.

The animal died on March 24th, 1910. Dissection showed that the ovaries had been nearly completely extirpated, with the exception of a few large ova and a few follicles. No regeneration of the ovaries had taken place. The thumb throughout the experiment remained unaltered.

No. 6.—Female frog, ovaries removed and two testes transplanted November 2nd, 1909. It was injected on March 3rd, 6th, and 10th with the substance of fifteen testes altogether. It remained healthy for two months after this, but died on May 25th, 1910. Dissection showed that the ovaries were completely gone, except two minute fragments which had not regenerated.

Throughout the experiment the thumb remained absolutely unchanged.

These two experiments may be taken as typical, since in none of the experiments on female frogs did any treatment result in any alteration of the thumbs to the male condition.

We may now turn to the effects of castration upon male frogs.

The effect of complete castration, both testes being removed, differs according to the time of year at which the operation is performed. If the frog is castrated during the breeding season the pads are very soon shed, and great difficulty may be found in keeping the animals alive. Thus—

No. 12.—Male frog, weighing 19 grm., with thumb-pads fully developed and pigmented, had both testes removed on March 5th, 1910.

On March 24th the external surface of the pads was completely shed and the surface was pale and comparatively smooth, though unpigmented papillæ of considerable size were still present on the pads.

No. 13.—Male frog, weighing 20 grm., with thumb-pads fully developed and pigmented, had both testes removed on March 5th, 1910. On March 25th the external layers of both pads were completely shed, and the surface was pale, and only showed comparatively small papillæ. The animal died on April 7th.

If castration is performed in the summer months, May to July, when the thumb-pads are normally small, comparatively smooth and unpigmented, the thumb-pads remain in this condition and do not develop the swollen glands or prominent papillæ the following autumn or winter. Thus—

No. 14.—Male frog, had both testes removed July 16th, 1909. At this time the thumb was smooth and unpigmented (fig. 23). It remained in this condition without any alteration until April 23rd, 1910, when it died.

If castration is performed during the autumn or winter months, when the thumb has begun to acquire its marked papillæ, glands, and pigmentation, the act of castration does not cause any disappearance of the papillæ or

Text-fig. D.

Camera drawing of outline of part of thumb-pad of male frog. No. 15, 5½ months after complete castration.

glands, but they remain in the condition in which they were when castration was effected for an apparently indefinite period. Thus—

No. 15.—Male frog, with moderately rough and swollen pads, was castrated on September 29th, 1910. Observations were made on this frog for a considerable time, but it was impossible to tell from inspection whether the pads decreased in size, or the papillæ were reduced. The animal was killed on March 9th, 1911, i. e. after a period of 5½ months, and drawings of part of the thumb-pad in a whole preparation (Text-fig. D) and of sections (fig. 24) are given. Marked papillæ are present, and a certain amount of pigment. The glands are swollen and well developed; in fact, the whole pad is in a condition normal for a September frog. There is no evidence that the pad has undergone any reduction, either in glands or papillæ, since September when castration was performed.

No. 16.—Male frog, with moderately rough, swollen and pigmented pads, was castrated on October 11th, 1910. No obvious change took place in the thumb, and the animal was killed on February 19th, 1911, i. e. after a period of four months. Sections (fig. 25) of the pad show very much the same condition as in No. 15; there is no evidence of the pad returning to the smooth reduced condition of the summer frog.

The effects of castration, therefore, are to make the thumb remain in much the same condition as it was when castration was effected, except in the case of the breeding spring-frog, when castration is followed by a rapid shedding of the pads.

We have now to follow the effects which ensue when a male frog is castrated and testes are inplanted into it, or the animal is injected with testis extract. Some of the animals used in this experiment have been previously described under the transplantation experiments.

No. 11.—Male frog, in which testes were severed and left in the abdominal cavity on October 4th, 1909. The thumb-pads at the time were somewhat pigmented, swollen, and covered with small papillæ, and while the frog was under observation the thumb-pads certainly did not shrink or become smoother or paler, and it was thought, though without much certainty, that these characters slightly increased. On February 10th, 1910, two testes from another frog were cut into pieces and inserted into the dorsal lymph-sac. On March 30th, 1910, the animal, being in good health and active, was killed, the severed and inserted testes were examined by microscopic sections, and the thumb-pads were also sectioned. The pieces of testis in the dorsal lymph-sac were undergoing disintegration and phagocytosis; the severed testes showed living spermatogonia, but disappearance of spermatozoa and replacement by fibrous tissue. The thumb (fig. 26) was in the condition of a normal autumnal frog, and there was no evidence that it had changed during the experiment.

No. 17.—Male frog, testes removed October 2nd, 1909. At this time the thumb-pads were rather small, slightly pigmented and rough, but not in so marked a degree as in No. 11.

On February 10th, 1910, two testes were cut up and inserted into dorsal lymph-sac. On March 3rd, 6th, 10th, 14th and 18th the animal was injected with testis extract, receiving in this time the substance of twenty-three testes.

The animal was killed on March 24th, 1910, being at the time in good health, and the thumb sectioned. The sections (fig. 27) show a condition of the thumb similar to that of a not very advanced autumn frog, and there is no evidence that the pad has undergone any increase in roughness, gland formation, or pigmentation since October, when it was castrated. Everything is consistent with the view that it has simply remained in the same condition without decreasing or increasing. The following two experiments were designed to test more accurately if injection of testis extract had any effect on the thumb. Unfortunately the animals died soon after the experiment was complete, so that some objection may be raised on the ground that the animals were not in good health.

No. 18.—Male frog, completely castrated on October 11th, 1910.

On October 20th, 1910, a piece of one of the thumb-pads was cut off and sectioned (fig. 28). It shows well-developed papillæ and glands. The frog was then injected on October 20th, 22nd, 25th, 27th, November 1st and 3rd with the substance of twelve fresh testes.

The pads during the injection showed no visible change.

On November 6th the animal died, and sections of the uninjured pad (fig. 29) showed no increase in papillæ, but an actual decrease in the size of the glands, probably owing to the ill-health of the animal.

No. 19.—Male frog, completely castrated on October 11th, 1910.

On October 20th, 1910, a piece of one of the thumb-pads was cut off and sectioned (fig. 30). It shows well-developed papillæ and glands.

On October 20th, 22nd, 25th, 27th, and November 1st, 3rd, 8th and 11th the animal was injected with the substance of sixteen fresh testes.

At no time did the thumb appear to undergo any change.

The animal died on November 16th, and sections (fig. 31) of the uninjured pad showed if anything a slight reduction of the papillæ owing to peeling of the superficial layers of skin. The glands, however, are very well developed—if anything, better than in the pad before the injections.

Results of Experiments on Thumb-pad.

The results of the above experiments show, firstly, that no amount of experimental treatment, such as ovariotomy, implantation of testes, injection of testis extract, has the slightest effect in making the female assume any of the male characters of the thumb. Castration in the male, we find, causes the thumb to be arrested in its development at the stage at which it was when castration was performed; it does not cause any immediate regression to the smooth condition even after a period of six months. This, however, only applies to autumn and winter frogs; breeding frogs in the spring, when castrated, very quickly cast off the external pigmented layers of the pads, though colourless papillæ of considerable size still remain on the pads.

The results we have obtained from implanting testes or injecting testis extract into castrated frogs have given wholly negative results, the thumb remaining in the state in which it was at castration.

We cannot pretend that our experiments on this head are complete, but they give us solid ground for criticising the conclusions which Nussbaum (**1**) and, later, Meisenheimer (**13**) have drawn from similar experiments, and our experience both with normal and experimental frogs makes us very sceptical as to the validity of the results claimed by these authors. In the first place, it is not possible to make reliable observations on the condition of the thumb-pad in the living frog by the mere inspection or feel of the pad without examining it carefully under the microscope. By mere inspection or feel of the pad an observer may consider the pad to be nearly smooth, when in reality papillæ of a fair size may be present. Then, later, at the close, perhaps, of an experiment, the pad may be examined under a lens or microscope, and the presence of the papillæ will probably be put down to the experimental treatment. Every gradation exists in the thumb-pads of male frogs at various periods, between the quite smooth and the exceedingly rough papillated condition of the breeding season, and the determination as to whether there has been an increase in papillation or pigment during the course of an experiment depends upon quantitative comparisons, which are difficult to make, and unless the state of the pad at the beginning of the experiment is very carefully determined, the end result of the experiment may be interpreted in a misleading manner. That the experiments of Nussbaum and Meisenheimer are unfortunately open to these objections is forced upon us by two principal facts: firstly, the changes which take place in the thumbs of the normal males have apparently not been quite correctly observed by these authors; and secondly, their belief that castration has the effect of causing a rapid reduction and disappearance of the papillæ is contradicted by our results. In the case of normal frogs (see Table, p. 468) we find that there is considerable variation in the development of the papillæ in different frogs at the same time of year, but a still more important fact is that in spring, after the breeding season is over and the external layers of the pad are thrown off, the thumb-pad does not at once return to a perfectly smooth condition, but remains papillated to about the same extent as a September or October frog. The attainment of the smooth condition is a gradual process, and is not completed until the late summer in the great majority of frogs. In the case of frogs castrated in autumn, when the papillæ have again begun to develop, we do not find that the papillæ are reduced as the result of castration, but that six months after castration the papillæ are in much the same condition as they were when castration was performed. Now, if we consider Nussbaum's and Meisenheimer's experiments in the light of these facts, also in the light of the entirely negative results obtained by us as the result of transplantation and injection of testis pieces or extract, we find that there is grave reason to doubt whether the positive results claimed by these authors are really conclusive.

Taking Nussbaum's experiments first, we may note that he is of opinion that castration may lead to a reduction in size of the papillæ, though this result, which is at variance with our own, is apparently obtained from mere inspection of the thumb at the beginning of the experiment, while at the close figures are given of microscopical preparations.

In two cases definitely positive results are claimed as the result of injecting castrated frogs with testis extract or of implanting pieces of testis in the dorsal lymph-sac.

In one case the frog was castrated on June 18th, when the pads are said to have been small and without papillæ. No drawing is given of the state of the pad at this time, and we suppose that the statement that the pad was without papillæ is based on inspection and not a careful examination under the microscope. In the other experiment the frog was castrated on May 26th, and a similar statement is made as to the state of the pad, but no drawings are given. During September and October both frogs were injected with testis extract or had pieces of testis implanted in them, and it is stated that the roughness on the thumb developed increasingly. As a proof that the growth of the papillæ is the result of testis injection, Nussbaum gives several figures, but in none of them is the condition of the pad before the experiment depicted, comparisons being instituted merely between the condition of the pad at the close of the experiment and that of a pad belonging to some other frog differently treated. Now without wishing to throw the least doubt on the good faith of these observations, we hold them to be insufficient, because there is no real evidence produced, apart from the mere inspection of the thumb at different times during the experiment, to show that small papillæ of the size of those shown in Nussbaum's figures, depicting the condition at the end of the experiment, were not present at the beginning of the experiment as well. We hold that the mere inspection of the thumb and a statement of the impression of the observer as to its state of papillation is not sufficient to prove a quantitative alteration in the degree of papillation such as is claimed in the experiments before us. The frogs were castrated in May and June, and it is stated that the pads were smooth. In our Table for normal frogs during May and June, half of the individuals had thumb-pads which on mere inspection might be described as smooth, but which on examination under the microscope revealed the presence of definite papillæ.

If such frogs were castrated there is nothing to show that the papillæ would disappear, as our evidence on castrated frogs goes to show that when frogs are castrated the thumb-pads remain *in statu quo* as regards the development of papillæ. Then on examining them in the following autumn a careful examination of the whole thumb, or of sections, would reveal the presence of papillæ, as in the case of Nussbaum's frogs, which were treated with testis injection and implantations.

Now let us turn to Meisenheimer's more recent observations. This author claims to have confirmed Nussbaum's results, but he has added to them in a remarkable manner, because he believes that he has induced the development of papillæ on the thumb-pad, not only by the implantation of testis-pieces, but also *by the implantation of pieces of ovary*. This truly amazing result is based on a number of experiments which appear to us quite unconvincing. Meisenheimer castrated his frogs in autumn or winter (September or January), and he states that the result of the operation "war eine sehr prompte, sie bestand in Uollen Ubereinstimmung mit Nussbaum's Ergebnissen in einer nahern völligen Reduktion der Daumenschwiele." This result, which is quite insufficiently supported by Nussbaum's experiments, is altogether at variance with our experiments Nos. 15 and 16, in which frogs were castrated in autumn, and after 5½ and 4 months respectively showed no reduction of the thumb-pads, while sections revealed the presence of marked papillæ (see figs. 28, 29). Meisenheimer gives drawings of the thumb of a male which had been castrated in September, 1909, and killed in October, 1910, but the drawing only refers to the thumb in October, 1910, and shows as a matter of fact the presence of small papillæ a year after castration, but no drawing is given of the same frog's thumb in September, 1909, to prove that the papillæ were really any larger then and that they had undergone reduction as the result of castration.

The same objection applies to the other experiments, and we are entirely unconvinced, especially when we take into consideration the results of our own experiments, that the result of castration in autumn or winter is to induce the complete reduction of the pad and its papillæ. This being the case, Meisenheimer's other results, namely, that in frogs castrated in autumn or winter the re-development of the thumb with its papillæ may be induced to some extent by the implantation of testes or ovaries, entirely fall to the ground. This supposed re-development of the papillæ is never, according to Meisenheimer, a complete one, and since no satisfactory evidence is produced to show that the papillæ ever disappeared as the result of castration, the presence of moderately developed papillæ in the castrated frogs when killed, after treatment with testes or ovaries, in the following autumn, may equally well be interpreted as the persistence of the original papillæ, as of the re-development of new papillæ under the influence of the testicular or ovarian transplantations.

It is interesting to observe that the figures given by Meisenheimer to support his contention fit in with our interpretation equally well as with his own.

Thus, in his figure 3, referring to a frog castrated in January, 1910, implanted with testes "in autumn," and killed on October 21st, 1910, the thumb is in the state of a normal January frog. In Fig. 4, referring to a frog castrated in September, 1909, implanted with ovaries "in autumn," and killed on October 21st, 1910, the papillæ on the thumb are far less developed than in fig. 3, and are in about the condition of a normal frog during September. This difference is attributed by Meisenheimer to the less effective action of the implanted ovaries compared to the testes, but according to our interpretation it is simply due to the fact that the frog referred to in fig. 3 was castrated in January, when the papillæ were comparatively well developed, while that referred to in fig. 4 was castrated in September, when the papillæ were less well developed. It is true that fig. 5, referring to a male castrated in January, is in an intermediate condition between figs. 3 and 4, but we know that the individual variability in the times and degrees of development of the papillæ in normal frogs is very great. Therefore, without fostering any preconceived hostility to the theoretical interpretations of these authors, or without in any way casting a doubt on their perfect good faith, we are utterly unable to regard the conclusions they draw from their experiments as in the least degree convincing. Any active influence on the thumb, either of testis injections or the implantation of pieces of testis or ovary, is according to our view entirely unproven.

The fundamental point in which these authors appear to have been mistaken is in supposing that the castration has the effect of always reducing the papillæ present on the thumb, and of causing them to disappear. The effect of castration, except actually during the breeding season, is to make the papillæ on the thumb-pads remain essentially in the condition in which they were at the time of castration. The presence of functional testicular tissue is necessary for the normal cycle of changes in the thumb-pad, and an essential part of this normal cycle is the acquisition of the absolutely smooth unpapillated condition of the summer frog. It is entirely unfounded to regard this smooth condition as the reduced phase to which thumbs are reduced as the result of castration; rather must it be regarded as an active phase brought about by the proliferation of the epidermis between the papillæ, which causes the depressions to be filled up and the papillæ to disappear. It is surely not without significance that this acquisition of the smooth thickened epidermis on the thumb coincides with the period of the greatest activity of the testes, when they are attaining their full size and are engaged in the maturation of the genital products (see Table, p. 468). The conclusion which we draw from the whole series of experiments, including our own and those of other authors, is that the cycle of changes in the thumb of the male frog is conditioned by the presence of testicular tissue in a normal functional condition, but that it has hitherto proved impossible to replace the action of the normal testicular tissue by means of the injection of testis-extracts, or by the implantation of testes or pieces of testis from other frogs. The fate of such introduced tissues is invariably degeneration, destruction and absorption, or replacement by fibrous tissue, and there is no experimental evidence, according to our view, to justify the conclusion that the introduced testicular matter, while it is being broken up and absorbed, gives rise to any substance which influences the secondary sexual characters in the same way as the presence of the normal, living testicular tissue of the individual itself.

The deduction, therefore, which has been widely based on Nussbaum's experiments, that the testis of the frog contains an internal secretion, which, on being circulated in the blood, calls forth the development of the secondary sexual characters, either with or without the mediation of the nervous system, is without experimental foundation.

The fact that the developmental cycle of the thumb depends for its normal course on the presence of normal living testicular tissue can be equally well explained on the theory that the testicular cells enter into a chain of metabolic processes in the body, which do not pursue their normal course in the absence of the testicular cells. This disturbance of the normal metabolic processes of the body, resulting in the failure of the metabolic organs of the body to give rise to their normal products in normal quantities, may have the result of inhibiting the further development of the secondary sexual characters. The development of these latter characters may depend, therefore, not directly on the action of an internal secretion or hormone derived from the gonad, but on the elaboration of other products in other organs of the body in their due proportion. These substances may be tentatively called "sexual formative substances," but we have no reason for supposing that they are entirely devoted to sexual or reproductive purposes, and that they take no part in the ordinary metabolic processes of the body. In the case of the female individual in birds and Crustacea, reason has been shown in preceding studies for associating the development of certain female secondary sexual characters with the elaboration and transportation of large quantities of fatty material. In the case of the male we do not know what the nature of the corresponding material may be, but it is an obvious suggestion that it is some substance capable of being worked up into chromatic or nucleo-proteid material, and giving rise to cellular proliferation, as opposed to the storing of reserve stuffs, such as fat. The exact mode in which the gonad conditions in certain cases the production of these formative substances in the required proportions is not known. The idea that the gonad acts upon the other organs of the body by means of an internal secretion which it gives to the blood is really based on the analogy of other organs of internal secretion, such as the thyroid, pancreas, etc., but we claim that there is no experimental basis for the idea, apart from analogy. The gonad may equally well exert its action by taking up some substance or substances from the blood, thus altering the composition of the blood, and perhaps stimulating the continued production of these substances in some other organ of the body. This explanation was the basal idea given to account for the action of Sacculina on crabs, and for the dependence of the comb-growth of hens on reproductive activity, and it accords with our results, on male frogs at least, equally as well as the theory of internal secretion.

Summary.

(1) The cyclical changes in the testes and thumb of male frogs are described. It is found that after the breeding season, although the outer papillated layers of the thumb-pads are thrown off, yet the pads do not immediately become smooth, papillæ of considerable size being present in the early summer months. The assumption of the perfectly smooth condition takes place gradually during the summer, and is due, not to a process of reduction, but to a prolifera-tion of the epidermis which fills in the valleys between the papillæ.

(2) Transplantation of testes into other individuals, whether male or female (allo-transplantation), leads to the breaking-up and degeneration of the ripe spermatozoa and the testicular tissue, and its replacement by fibrous tissue, the greater part of which is derived from the host and invades the degenerate testis from the place of attachment to the host's body. Testes transplanted into the peritoneal cavity of another individual always become attached to the peritoneal lining and rapidly vascularised. Phagocytosis plays an important part in the absorption of the degenerate spermatozoa.

(3) When the testes are simply severed from their connections in the body and left unattached in the peritoneal cavity of the same individual (auto-transplantation), they soon acquire new attachments and vascularisation. The ripe spermatozoa rapidly degenerate and are finally replaced by fibrous tissue, partly, at any rate, derived from the testis itself, but the spermatogonia of the peripheral tubules survive, and proliferate in an active manner. This survival of the germinal cells in the case of auto-transplantation never occurs in allo-transplantation.

Reproductive Cycle in Normal Male Frogs.

Specimen.	Time of year.	Weight in gr.	Condition of thumb.	Condition of testes.
g. 1	April 18th	About 22	Pigmented pad of breeding-season has been shed, but marked colourless papillæ are present, giving pad a rough texture.	Sperm has been shed, testis is small. Only spermatogonia present, and remains of supporting cells.
g. 2	Ditto	About 20	Pads quite smooth, no papillæ to be observed.	
h. 1	May 9th	About 20	Practically smooth, a trace of papillæ which hardly project above general surface.	Ditto.
h. 2	Ditto	About 18	Papillæ well marked, nearly as in g. 1.	
j. 1	June 20th	27·5	Papillæ fairly well marked, not so large as in g. 1 or h. 2.	Testis increased in size; marked formation of masses of spermatocysts.
j. 2	,,	21	Practically smooth.	
j. 3	,,	16	Slight papillation.	
j. 4	,,	14	Smooth.	
j. 5	,,	10	,,	
k. 1	July 11th	24	,,	Active multiplication of spermatocysts.
k. 2	,,	22	,,	
k. 3	,,	14	,,	
k. 4	,,	7·5	,,	
l. 1	Aug. 1st	16·5	Smooth; marked increase in glands.	Spermatogenesis in full process. Spermatids present in places. Testes very large.
l. 2	,,	15·5	Very slight papillæ.	
l. 3	,,	12·5	,,	
l. 4	,,	8·5	Smooth.	
l. 5	,,	4	,,	
m. 1	Aug. 22nd	21	Very slight papillæ.	Spermatids and ripe spermatozoa in many tubules.
m. 2	,,	26	Papillæ marked.	
m. 3	,,	19	Smooth.	
m. 4	,,	17	,,	
m. 5	,,	15	Very slight papillæ.	
m. 6	,,	10·5	Smooth.	
n. 1	Sept. 19th	25	Papillæ well marked.	All tubules full of fully formed spermatozoa. Absence of any other stages, except spermatogonia lining tubules. Spermatogenesis definitely finished.
n. 2	,,	22·5	Papillæ highly marked.	
n. 3	,,	20	Papillæ marked.	
n. 4	,,	16	Papillæ highly marked.	
n. 5	,,	12·5	Papillæ marked.	
n. 6	,,	9	,,	
o.–u.	Oct.–March	—	Continued increase of papillæ up to breeding period.	Testes remain unaltered, until shedding of sperm.

(4) Ovariotomy, with or without subsequent implantation of testes or injection of testis-extract, has no effect in making the thumb of the female assume any male characters.

(5) Castration in the male, except during the breeding season, does not have any marked effect in reducing the papillation of the thumb, the papillæ in a castrated frog remaining for a long period (at any rate, four to five months, and probably much longer) in the same condition as they were at castration. Castration during the breeding season is followed by a rapid throwing-off of the outer papillated layers of the thumb-pads.

(6) Implantation of testis-pieces or of whole testes, or the injection of testis-extract, has an entirely negative effect on the thumb of castrated frogs, and does not cause any appreciable increase of the papillæ.

(7) The positive results claimed by Nussbaum and Meisenheimer, as following the treatment of castrated frogs by the above and similar means, are criticised and shown to be altogether unproved. There is, therefore, no evidence that the testes of the frog contain an internal secretion which, on being injected into a castrated frog, calls forth the increase of the papillæ on the thumb.

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EXPLANATION OF PLATES 43–46,

Illustrating the paper by Mr. Geoffrey Smith and Mr. Edgar Schuster on "Studies in the Experimental Analysis of Sex."

PLATE 43.

Fig. 1.—Experiment No. 2. Section through testis. × 21. *bl.* Blood vessels. *fi.* Band of fibrous tissue. *tu.* Degenerating tubules.

Fig. 2.—Experiment No. 3. Section through testis. × 21.

Fig. 3.—Experiment No. 3. Small portion of testis. × 545. *phag.* Phagocytes. *sp.* Spermatozoa appearing to break up into chromatin granules.

Fig. 4.—Experiment No. 4. Section through testis No. 1. × 21.

Fig. 5.—Experiment No. 4. Section through testis No. 2. × 21. *fi.* Fibrous tissue seen to have invaded the tubules and replaced remains of spermatozoa. *pi.* pigment.

Fig. 6.—Experiment No. 4. Testis No. 2, a portion, × 199, showing degenerating tubules.

Fig. 7.—Experiment No. 4. Testis No. 1, a portion. × 250. fib_1. Fibrous tissue indicating the walls of tubules. fib_2. Fibrous tissue which has grown into the tubules, and has replaced, or is replacing, the remains of spermatozoa (*sp.*).

Fig. 8.—Experiment No. 6. Section through testis and the tissue to which it is attached. × 21. *tu.* The remains of two tubules. *pi.* Pigment.

Fig. 9.—Experiment No. 6. Small portion of testis. × 250. Showing part of the remains of one tubule and surrounding tissue.

PLATE 44.

Fig. 10.—Experiment No. 7. Section through testis. × 21. *bl.* Blood-vessels. tub_1. Tubules showing incipient stages of degeneration. tub_2. Tubules showing later stages of degeneration. *fi.* Fibrous band.

Fig. 11.—Experiment No. 7. Tubules showing incipient stages of degeneration. × 199.

Fig. 12.—Experiment No. 8. Section through testis. × 21. *bl.* Blood-vessels.

Fig. 13.—Experiment No. 9. Section through testis. × 21.

Fig. 14.—Experiment No. 10. Section through testis No. 1. × 21. *bl. sp.* Large blood-spaces, occupying more than half the area of the section. *tub. dg.* Degenerating tubules. *tub. rg.* Regenerating tubules.

Fig. 15.—Experiment No. 10. Section through testis No. 2. × 21. *fib.* Central mass of fibrous tissue. *tub. rg.* Tubules lined with living and proliferating spermatogonia.

Fig. 16.—Experiment No. 10. Part of testis No. 1. × 199. Showing blood-spaces (*bl. sp.*), degenerating (*tub. dg.*) and regenerating tubules (*tub. rg.*).

Fig. 17.—Experiment No. 10. Small regenerating tubule from testis No. 1. × 545.

Fig. 18.—Experiment No. 10. Part of central fibrous portion of testis No. 2. × 545.

PLATE 45.

Fig. 19.—Experiment No. 10. Testis No. 2. Tubule from the end of testis lined with actively proliferating cells. × 545.

Fig. 20.—Experiment No. 11. Testis No. 1. × 21. fib_1. Fibrous surrounding layer containing large quantity of pigment (*pi.*). fib_2. Central fibrous portion. *tub.* Regenerating tubules.

Fig. 21.—Experiment No. 11. Testis No. 1. A portion from near the periphery. × 250. Lettering as above.

Fig. 22.—Experiment No. 11. Testis No. 2. × 21. *bl.* Blood-vessel. *tub. reg.* Regenerating tubules. *tub. dg.* Degenerating tubules.

PLATE 46.

Sections of thumb-pads. × 67.

ep. Epidermis. *gl.* Glands. *pi.* Pigment.

Fig. 23.—Experiment No. 14.
Fig. 24.—Experiment No. 15.
Fig. 25.—Experiment No. 16.
Fig. 26.—Experiment No. 11.
Fig. 27.—Experiment No. 17.
Fig. 28.—Experiment No. 18 on October 20th, 1910.
Fig. 29.—Experiment No. 18 on November 6th, 1910.
Fig. 30.—Experiment No. 19 on October 20th, 1910.
Fig. 31.—Experiment No. 19 on November 16th, 1910.
Fig. 32.—Normal frog (*l.*) killed in August.